

POPULATION GENETICS OF GENE FUNCTION

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Abstract

This paper shows that differentiating the lifetimes of two phenotypes independently from their fertility can lead to a qualitative change in the equilibrium of a population: since survival and reproduction are distinct functional aspects of an organism, this observation contributes to extend the population-genetical characterisation of biological function. To support this statement a mathematical relation is derived to link the lifetime ratio T_1/T_2 , which parametrizes the different survival ability of two phenotypes, with population variables that quantify the amount of neutral variation underlying a population's phenotypic distribution.

During the last decade experimental research has begun using population-genetical principles to link the function of genes to their population distribution [12, 15, 14]. This closely parallels the way in which statistical thermodynamics links the macroscopic state of a physical body to the activity of the molecules that form it, and is likely to bear similarly significant consequences.

Population genetics has studied the mathematical relation of evolutionary and demographic forces to the population distribution of alleles for more than a hundred years, and bioinformatics has long used conserved sites in comparative statistical data to infer function [12]. More recent works can therefore be seen as a joint maturation of these long-standing research threads, which enables to pose questions about the relation of gene function to gene distribution in a more detailed manner than previously possible: the analogous maturation which gave birth to statistical thermodynamics consequently allowed to infer the size of a molecule from observable dynamics.

One important point that has been stressed in recent works is that demographic forces can mimic the effect of selection during evolution, so that it is necessary to account

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for demography when inferring selection from population statistics. In [15] the authors consider the effect of a changing population size, and show that a population's pattern of variation may be used to infer this type of demographic information, as well as to locate functionally important genetic sequences.

The present paper considers a further possibility granted by this approach, which lies in using the different effects of demography and reproductive selection on gene distribution in order to infer more detailed information about gene function itself.

It needs to be stressed that, for the sake of exposition, this paper uses the term "gene function" as relating to a univocally defined concept, while yet acknowledging that this concept clearly doesn't exist as such. One good way to justify such simplistic use lies in the analogy with the term "the meaning of a word", which similarly can sometimes be put to good use, while suffering from an analogous ambiguity of definition.

Life-expectancy, a demographic parameter, is related to function: it reflects, statistically, the ability of an organism to perform the tasks which are required in order to survive for certain amount of time. If life-expectancy is systematically different for two phenotypes in a given environment, it is natural to conclude that this difference is due to the different way in which the two phenotypes function in such environment.

Therefore, if we consider survival and reproduction to be two macro-functions performed by any living organism, detecting a difference in phenotypic life-expectancy - separately from a difference in fertility- must allow to get a two-dimensional description of the gene function which underlies the phenotypic change. This suggests that including the effect of demographic forces may not only be used to infer information about a species' demographic history, but also to get more detailed information regarding the specific function that a gene is playing in a given environment, using only information which characterises the population as a whole.

This paper shows that life-expectancy can affect the nature of a population's phenotypic distribution in a way which is qualitatively distinct from the effect of fertility. It is also shown that if a population contains two phenotypes characterised by different

life-expectancies T_1 and T_2 , then the ratio

$$\lambda = \frac{T_1}{T_2}$$

can be quantitatively estimated through the phenotypes' respective amounts of neutral variation, independently of all other parameters.

Since a population's dynamics is typically dominated by differences in fertility, standard models tend to combine the effect a genetic change has on an organism's survival ability with its effect on fertility [4, 11]. In this paper we study the effect of a difference in survival explicitly: we find that this effect is indeed small, but that it leads to qualitative changes in a population's equilibrium regime, which are likely to generate statistically observable signals at a population as well as at a comparative level.

The paper is organised as follows: in the next section we describe the empirical justification for the structure of the chosen model, and we then present the model in section 2. Our modelling framework consists of two levels: one level studies the equilibrium reached by two available phenotypes, and is analysed in section 3. It is at this level that we observe a qualitatively novel equilibrium regime that results from differentiating the phenotype lifetimes separately from their reproductive rates.

Section 4 focuses on the second level of description, which corresponds to the amount of neutral variation characterising each of the two considered phenotypes. We are interested in this variation because it allows to express the parameter $\lambda = T_1/T_2$ in terms of statistically observable data.

We conclude the paper by considering the practical limitations of the derived results and briefly outlining possible further developments.

1 Empirical background

We want to construct a stochastic model for the dynamics of a population consisting of two phenotypes which differ both in their average amount of offspring (which we call W_1 and W_2 , respectively), and in their average lifetimes (T_1 and T_2). To this end, in section 2 we modify the haploid Moran model by a qualitative change in its process, which we

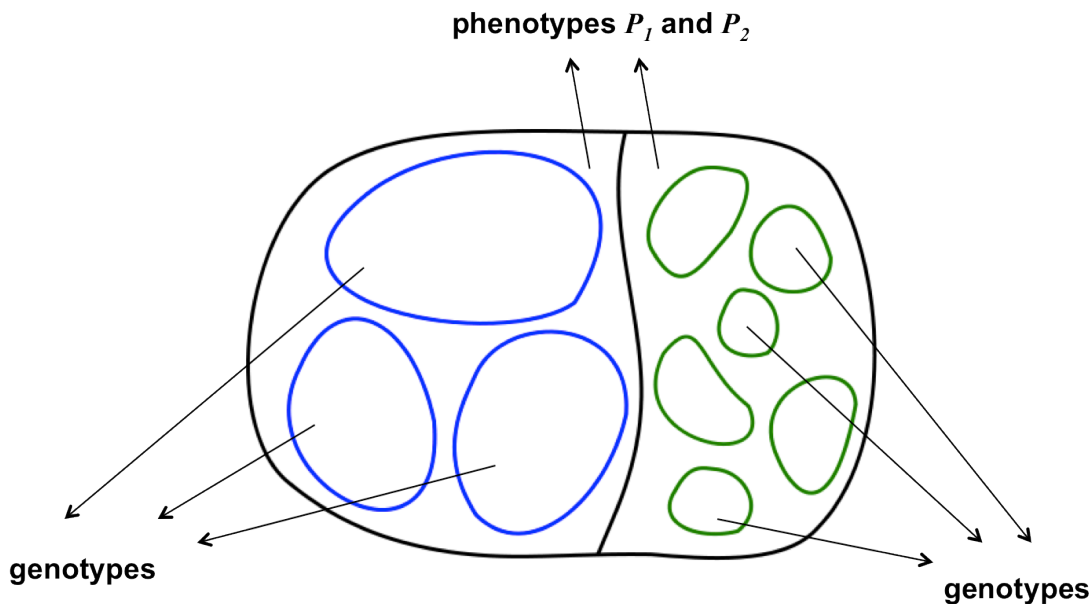


Figure 1: *Structure of the considered population: organisms carry one of two phenotypes, P_1 and P_2 , each of which is coded by many synonymous genotypes that are assumed to be neutral with respect to each other.*

attempt to justify on intuitive grounds, and which we parametrize in terms of a novel parameter, $\lambda = T_1/T_2$.

Since the model includes one more parameter than the standard setting, it is desirable to correspondingly expand the number of independent quantities that we expect to observe, so to allow the discrimination among possible causes of specific states of the population. We address this need by considering the amount of synonymous variation included in each of our two phenotypes, which, following [15], we consider to be neutral: we therefore have a population consisting of two phenotypes coded by a larger number of genotypes, which are neutral with respect to each other as long as they give rise to the same phenotype (Figure 1).

This population structure parallels the structure of variation encountered by Martin Kreitman when studying the alcohol dehydrogenase locus in *D. melanogaster*, where he found that only one of the 43 polymorphic sites observed in his sample led to a change in the protein coded by the gene, thus revealing that the gene consists of only two molecular

phenotypes coded by a larger number of genotypes [9].

Though the model we consider is clearly much too simplistic to apply to a natural population of *Drosophila*, Kreitman's observation gives empirical justification for building a model which allows only one of the sites of a long genetic sequence to lead to a change in phenotype, which would a priori seem to be a very strong assumption.

We will see that by using such simplification -which is further supported by more extensive studies [1]- our model allows both to derive a clear characterisation of the phenotypic equilibria (section 3), and to estimate the model's novel parameter λ (section 4) in terms of statistically observable quantities, by using variations of standard results.

2 Modelling approach and its relation to the standard setting

The structure of our model reflects the empirical situation described in the last section: we consider a population of N organisms carrying genotypes of length L , where each genotype-site can take two possible states: however, only one of these sites corresponds to a change of phenotype, whereas mutations in other sites are neutral. It is worth pointing out that this is the same structure of the *Drosophila* haplotype data included in the appendix of [1], where only mutations in one specific molecular-marker site lead to an amino acid change.

The model therefore includes two levels of description: a phenotypic one, which describes the dynamical change in the number of individuals carrying the two available phenotypes, and a genotypic level that characterises the amount of neutral variation available for each of the two phenotypes.

We use the symbols P_1 and P_2 to denote our two phenotypes, and we keep the population size fixed at a value N : we can therefore specify the phenotypic state of the

population by a random variable X which gives

$$\begin{aligned} X &: \text{ number of individuals with phenotype } P_1, \\ N - X &: \text{ number of individuals with phenotype } P_2. \end{aligned}$$

We are interested in studying the stochastic equilibrium reached by a process of death, reproduction and reversible mutation, where mutation happens with the same probability u in both directions, and for all the available sites, including the phenotypically-linked one.

The assumption of a mutation rate which is both symmetric and site-independent is very idealised, and even more importantly, an equilibrium due to reversible mutation is not generally considered to be relevant for generic mutations [7].

Due to its simplicity, however, the chosen setting allows to show with remarkable clarity that an explicit difference in the phenotype lifetimes leads to a qualitative novel type of equilibrium state: this is the aim of this paper, since it is in this novelty that we see potential to extend the standard population-genetical characterisation of biological function; having a full characterisation of this elementary case should be useful when considering more realistic and analytically challenging situations.

In the next subsection we therefore describe a variation of the Moran process which, as we will attempt to justify on intuitive grounds, provides a good model for phenotypes that are allowed to differ independently in lifetime and average number of offspring. It is interesting to point out that Moran introduced in Ref. [11] a variation of his original model that implements reproductive selection by differentiating his alleles' lifetimes while keeping the instantaneous reproductive rates the same for all his phenotypes (thus differentiating their life-long reproductive yields): Moran remarks that considering lifetime and reproductive differences separately would *almost certainly* make no difference for the equilibrium distribution. Our implementation of the phenotypic difference comes as a natural extension of Moran's, and our claim is that, though the subtlety of this extension's effect roughly confirms his intuition, the qualitative nature of this change provides considerable descriptive potential.

As stressed before, when introducing a quantity λ to parametrize the differentiation in the lifetimes, it becomes desirable to extend the set of quantities that we expect to observe in the statistics of the population: in other words, though the quantity

$$x = \frac{X}{N}$$

fully characterises our population's phenotypic state, we can gather further information by looking at how the X individuals carrying phenotype P_1 are partitioned into synonymous genotypes, and similarly for the $N - X$ individuals carrying phenotype P_2 .

A natural intuitive choice to characterise neutral variation in the two phenotypes would be to count the actual number of synonymous genotypes present for each: however, as remarked in [4], an interesting alternative is to use the inbreeding coefficient concept.

The inbreeding coefficient is typically used for diploid organisms, since it is defined as the probability that a given genetic locus is homozygous: that is, it is the probability that for a diploid organism chosen at random from a population, the two alleles that the organism contains at such locus are found to be identical. However, under the assumption of random mating, this turns out to be equivalent to the probability that any two alleles drawn at random from the population are identical, regardless of the separation into organisms.

The latter definition makes the quantity relevant to haploid populations, and it turns out to be a more analytically accessible one than the aforementioned *actual number* of synonymous genotypes, at least for the estimation purpose considered in section 4, in which we generalise a result taken from [8]. The inbreeding coefficient also provides an effective approximation to the actual number of synonymous alleles, and it has been suggested to be more empirically accessible than the latter quantity [4].

2.1 Phenotypic level

Here we describe the process through which we model the change in our population's phenotypes, and make our attempt to justify the modelling choice through intuition: the observable outcome of this process is analysed in section 3.

Studies using population genetics to infer function are typically based on the Wright model [12, 15], which describes the stochastic change of a population at discrete non-overlapping generations, and it is customary to describe the allele dynamics by using a continuous approximation to this process. There is, however, a somewhat paradoxical aspect to this standard modelling approach: whereas the Wright model describes the population as changing in discrete generations which might last for a considerable amount of time, the continuous approximation requires such generations to be taken of vanishing duration.

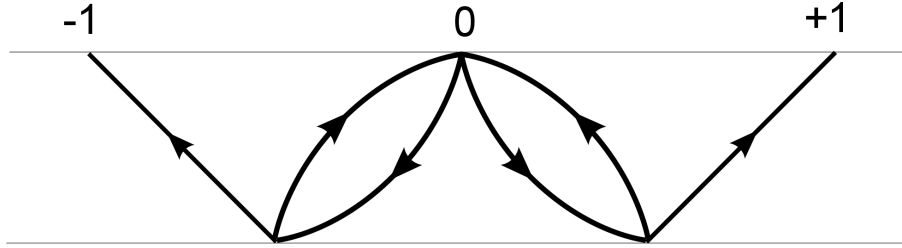
This assumption is well justified by the fact that processes are often considered to be taking place on an evolutionary time scale, which is much longer than the generation time, as well as by the fact that if one assumes organisms not to be subject to ageing, which is virtually unavoidable at the simplest level of description, the Wright model is formally equivalent to a process of death and birth [3].

On the other hand, the point of view of this paper is that, though suitable given the typical assumptions, the reliance on a discrete generation setting might hinder the consideration of relevant modifications: here we propose a modification to the Moran model that stems from features of an instantaneous event, which are prohibitively difficult to visualise at a generation level.

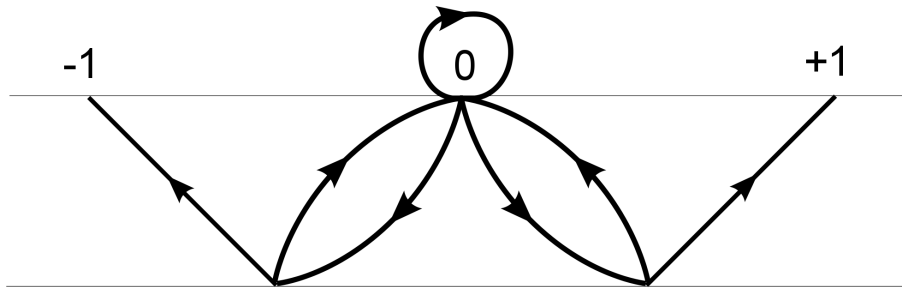
The Moran model describes the process of change in a population consisting of two types of organism. A time interval in this model is defined by the occurrence of a death event, followed by a birth event. It is sensible, in principle, to define time through these events, since these change the state of the population, which is the object under study.

There is a reason, however, to regard time as flowing according to an external frame of reference, thus including time intervals during which no population events happen: death events may be thought to take place at a rate determined by the physiology of the organisms; due to competition, however, birth events may be thought to happen instantaneously when the death of an individual makes a safe spot available in the environment.

In fact, both the Wright and the Moran model set the population size to be fixed at a value N , which represents the environment's carrying capacity: the meaning of this



(a)



(b)

Figure 2: Here we illustrate the difference between the (a) Moran process and (b) the process considered in this paper. At every time interval in (a) an organism is chosen to die, and a new one is chosen to replace it: the types of the dead and newborn organisms determine change in phenotype P_1 's frequency X as -1 , 0 or 1 . The loop in (b) shows that in the present model time flows according to an external reference: this allows to characterise the different nature of birth and death events.

constraint is that a death event should be interpreted as one which vacates an environmental safe spot, and we argue that the presence of competition makes it intuitively admissible to model the subsequent birth event as happening instantaneously as soon as the environmental spot becomes available.

As a consequence, having an external time reference allows to describe more adequately the interplay between the two different types of competition which characterise 1) an organism’s struggle to survive, and thus to preserve its environmental spot, and 2) the reproductive struggle to occupy all spots as soon as they become available.

According to this interpretation, all organisms in a population might be thought to be playing a waiting game similar to the children’s game “musical chairs” where $N - 1$ chairs are available for N children to sit on when the music stops. This process has already been considered in an evolutionary setting in [2]: in our case, rather than focusing on modelling the competitive game which determines the allocation of an available spot, we consider this allocation to happen trivially and instantaneously, and we focus on the waiting game itself.

In practice, our modification of the Moran model consists of a process which allows *at most* one death-birth event per time interval rather than *exactly* one as in the original model [10]: the two diagrams in Figure 2 illustrate this difference.

Figure 2(a) shows the change in the phenotype frequency X during a time interval in the original Moran model: an individual is chosen at random from the population and killed, and then replaced by a new individual. The change in X is then determined by the phenotypic identity of the dead and of the newborn.

Differently from Figure 2(a), Figure 2(b) (which describes our process) contains a loop at the origin of the diagram. This formalises the different nature of the death and birth events: at a given time instant no organisms might die; when a death does happen, however, a birth systematically follows instantaneously.

This modelling choice is arbitrary: contrarily to our assumption, following a death competitive conflicts between organisms might lead to a substantial delay in the allocation of the newly-vacated spot, and this could considerably change the nature of the process.

This objection, however, only highlights the descriptive potential of a modelling approach that uses intuition to consider fundamental population events in some detail, an approach for which a considerable gap exists in the mathematical biology literature: here we look at the consequences of a simple such possibility.

The model is therefore defined by the transition probabilities p^- , q^- , p^+ and q^+ in Figure 3, which are in turn derived from the life-cycles of the two organisms.

Transition probability p^- corresponds to the event that an organism with phenotype P_1 dies, whereas q^- corresponds to the same event for phenotype P_2 .

We denote the relative frequency of phenotype P_1 by $x = X/N$, and its average lifetime by T_1 : under the assumption that each organisms is reproductively mature at birth and is not subject to ageing, we have that

$$p^- = \frac{x}{T_1}, \quad \text{and} \quad q^- = \frac{1-x}{T_2}. \quad (2.1)$$

In this paper we refrain from giving a fully detailed derivation of these formulas: the issues involved in the rigorous foundation of this level of modelling are problematic, and this is indeed related to the fact that variations such as (2.1) are not often encountered in the literature. This paper rather tackles foundational issues by proposing (2.1) as a specific variation of the standard approach.

The technical aspects of the derivation of (2.1) are not, however, fundamentally different from those encountered in the Wright and the Moran models, and the connection can be intuitively clarified by the following observation. The quantity p^- is the product of 1) the Moran-like probability that an organism carrying phenotype P_1 is chosen to die ($x = X/N$), and 2) the probability that it *actually* dies. The latter probability, which we can call δ_1 , corresponds to the fact that in our model organisms are always given a chance to survive: this can be given a more fundamental justification if one considers a model in which an arbitrary number of organisms can die at any given time interval. We shall, however, refrain from pursuing this line of reasoning further, and leave it for a more specific future work.

We want our model's parameters to correspond to biological features: assuming that our organisms are not subject to ageing, or to environmental fluctuations, we have that

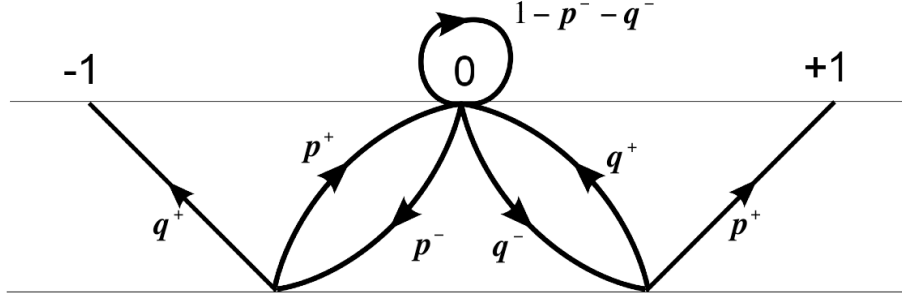


Figure 3: *Transition probabilities for the fundamental population events in our process: p^- and p^+ correspond to death and birth (after mutation) of organisms with phenotype P_1 . Similarly we have q^- and q^+ for P_2 , and the existence of the loop at the origin of the diagram is due to that in general $p^- + q^- < 1$.*

their average lifespan is equal to the mean of a geometric distribution with parameter δ_1 (for phenotype P_1), which leads to

$$T_1 = \frac{1}{\delta_1}.$$

This gives p^- in (2.1), and the same reasoning applies to q^- .

In view of (2.1) we have that in general

$$p^- + q^- < 1,$$

and this is the cause of the qualitative effect arising from differentiating the phenotypic lifespans, which gives the model's novelty.

The second biological feature which we assign to our phenotypes is the average number of offspring produced by an organism during its entire lifetime, which we denote by W_1 and W_2 for phenotypes P_1 and P_2 , respectively.

Transition probabilities p^+ and q^+ are obtained by considering elementary events in a similar way as for p^- and q^- , taking into account that reproduction involves also mutation, which we model as happening with probability u in both directions.

Under these life-cycle conditions it can be shown that

$$p^+ = \frac{\frac{W_1}{T_1} (1-u)x + \frac{W_2}{T_2} u(1-x)}{\frac{W_1}{T_1} x + \frac{W_2}{T_2} (1-x)},$$

and

$$q^+ = \frac{\frac{W_1}{T_1} ux + \frac{W_2}{T_2} (1-u)(1-x)}{\frac{W_1}{T_1} x + \frac{W_2}{T_2} (1-x)}.$$

The reason for the denominators in p^+ and q^+ is that, as we stressed before, a reproduction event is assumed to happen instantaneously when a death event vacates an environmental spot, so that a “death followed by no birth” is not considered to be a possible event. This determines the “musical-chairs” nature of the model, which we claim to be a particularly insightful way of modelling a process of competition [2], and which quantitatively corresponds to

$$p^+ + q^+ = 1.$$

We are particularly interested in including parameter values for which the equilibrium distribution does not become trivial in the limit of large population size, and to this end we employ the following asymptotic scalings for the mutation parameter

$$Nu \xrightarrow{N \rightarrow \infty} \theta,$$

and for reproductive selection parameter

$$N \left(\frac{W_1}{W_2} - 1 \right) \xrightarrow{N \rightarrow \infty} s,$$

which we use as definitions for the rescaled parameters θ and s . For convenience we also use the parameter λ for the ratio between lifetimes:

$$\lambda = \frac{T_1}{T_2}.$$

In section 3 we will need the first two moments of the change in the variable X at a given time to write down the large population size limit for the equilibrium distribution attained by the phenotypes. To this end, after defining

$$\Delta X(x) = X_{t+1} - X_t,$$

we need to compute quantities $M(x) = \mathbb{E} [\Delta X(x)]$ and $V(x) = \mathbb{E} [(\Delta X(x))^2]$ in terms of the transition probabilities defined above. The functional dependence on x shows that this moments are computed conditionally on the relative frequency of phenotype P_1 being equal to $x = X/N$: for convenience, however, from now on we drop the x dependence from the notation.

Using inspection on Figure 3 we find that

$$M = \lim_{N \rightarrow \infty} (q^- p^+ - p^- q^+),$$

and that

$$V = \lim_{N \rightarrow \infty} (q^- p^+ + p^- q^+),$$

which in terms of the asymptotic parameters gives

$$M = \frac{1}{NT_1} \cdot \frac{\theta \lambda^2 (1-x)^2 + \lambda s x(1-x) - \theta x^2}{x + \lambda(1-x)},$$

and

$$V = \frac{2\lambda}{NT_1} \cdot \frac{x(1-x)}{x + \lambda(1-x)}.$$

We see that the novelty of the model is nicely shown algebraically by the presence of a factor $(x + \lambda(1-x))$ in the denominator of both M and V , its presence in the latter being particularly significant for the form of the equilibrium distribution: we discuss the analytic consequences of this in section 3.

2.2 Neutral variation

Underlying the process of change in the phenotypic frequencies we have the process of creation of new neutral mutations to phenotypes P_1 and P_2 , and of their stochastic loss.

As mentioned in the introduction to this section, we model each genotype as a sequence of L two-state sites, which includes a site (the “phenotypically-linked” site) whose mutation causes the change between phenotypes P_1 and P_2 , and for the sake of simplicity we make the rather strong assumption that mutation happens with same probability u at all sites, and in both directions: therefore, the probability of mutation u relevant to

the phenotypic equilibrium also parametrizes the amount of neutral variation for the two phenotypes.

Like we said before, rather than using the actual number of neutral genotypes into which each phenotype is partitioned, we choose to characterise neutral variation by the inbreeding coefficient. For a population of haploids, such as the one we consider, the inbreeding coefficient can be defined as the probability that two genotypes drawn at random from the population are identical.

Therefore, in addition to random variable x that characterises the population’s phenotypic distribution, we define the two quantities

F_1 = probability that two organisms with phenotype P_1 have the same genotype,

F_2 = probability that two organisms with phenotype P_2 have the same genotype.

In section 4 we find an explicit formula for the new parameter λ in terms of combined moments of quantities F_1 , F_2 and x : in this paper’s point of view such a relation contributes to extend the population-genetical characterisation of biological function.

Kimura and Crow [8] find that in an “infinite alleles” model, which compared to the model presented here may be thought of as one where only one phenotype exists, and where genotypes have an infinite number of sites, the inbreeding coefficient is on average equal to

$$\langle F \rangle \approx \frac{1}{2Nu + 1}.$$

In section 4 we generalise their calculation to include our case, and see how the result can be used to express the parameter $\lambda = T_1/T_2$ in terms of statistically observable quantities.

3 Phenotypic equilibrium

Here we describe the equilibrium distribution attained by the population’s phenotypes P_1 and P_2 under our process of selection and reversible mutation. It is worth stressing immediately that the qualitative novelty of including the differentiation of phenotype lifetimes manifests itself analytically in the probability density function of P_1 ’s relative

frequency x ,

$$\phi(x) = C e^{\alpha x} x^{\lambda\theta-1} (1-x)^{\theta/\lambda-1} (x + \lambda(1-x)),$$

through the factor $(x + \lambda(1-x))$, which is not usually seen in population genetics models. When $\lambda = 1$ this factor is equal to one, and we recover the typical equilibrium distribution for a haploid population under reversible mutation and selection.

3.1 Form of the equilibrium distribution

The form of the phenotypic equilibrium distribution can be obtained in a large population size approximation by using Wright's formula

$$\phi(x) = \frac{C}{V(x)} \exp \left\{ 2 \int \frac{M(x)}{V(x)} dx \right\}, \quad (3.2)$$

where C is a normalisation constant.

This formula was derived by Wright [16] for a process divided into non-overlapping generations, and it has been proved by Moran to be applicable to various versions of his model [11]. More importantly for our case, Cannings [3] has shown by a concise observation that an overlapping generations model can be considered formally equivalent to a non-overlapping one as long as organisms are not subject to ageing, and this allows us to use approximation (3.2) in its full generality.

According to the last section our model gives

$$M = \frac{1}{NT_1} \cdot \frac{\theta \lambda^2 (1-x)^2 + \lambda s x(1-x) - \theta x^2}{x + \lambda(1-x)},$$

and

$$V = \frac{2\lambda}{NT_1} \cdot \frac{x(1-x)}{x + \lambda(1-x)},$$

so our model's equilibrium ϕ for P_1 's relative frequency $x = X/N$ takes the following form

$$\phi(x) = C e^{\alpha x} x^{\lambda\theta-1} (1-x)^{\theta/\lambda-1} (x + \lambda(1-x)),$$

where

$$\alpha = s + \theta \left(\frac{1}{\lambda} - \lambda \right),$$

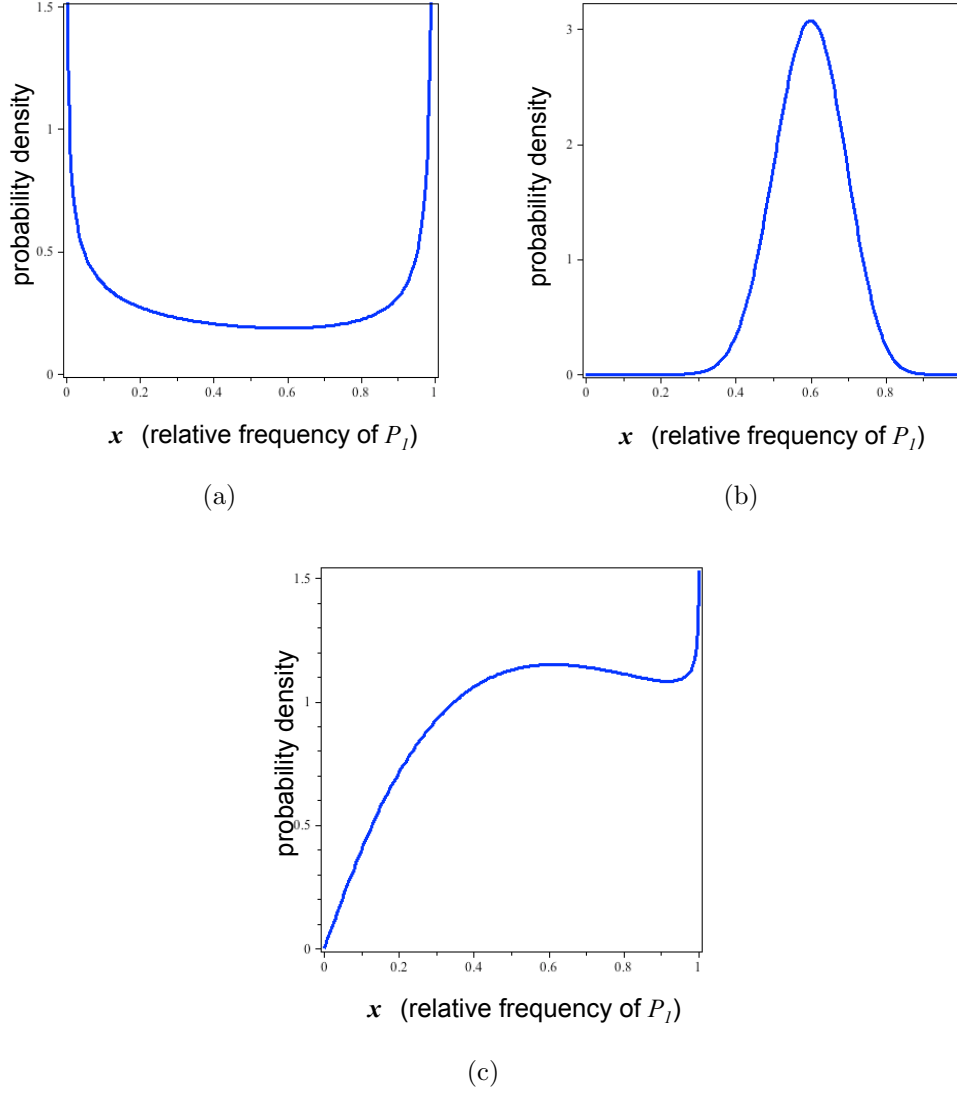


Figure 4: *These are the three basic types of equilibrium distribution which can be attained at a phenotypic level, and which correspond to different intensities of mutation in the following way: (a) $Nu \ll 1$, (b) $Nu \gg 1$, (c) $Nu \approx 1$. Regime (c) is caused by the differentiation of the phenotype lifetimes, and appears when $\lambda \neq 1$.*

and C is the normalization constant which ensures that

$$\int_0^1 \phi(x) dx = 1.$$

We see that for $\lambda = 1$ (that is, when $T_1 = T_2$) we recover the equilibrium distribution for a typical haploid random drift model with reversible mutation. When $\lambda \neq 1$ we have that the factor $(x + \lambda(1 - x))$ produces a qualitative difference in the shape of the distribution, though this only happens for values of $\theta = Nu$ close to one: Figure 4 shows the shape of the equilibrium distribution for different values of the mutation parameter.

We find that there is an intermediate regime (Figure 4(c)) between the typical low-mutation U-shape (Figure 4(a)) and the high-mutation bell-shape (Figure 4(b)). The intermediate regime admits two stationary points, and as a consequence becomes bimodal. In general, we have that for any parameter value the type of equilibrium can be characterised in terms of the number of stationary points which the distribution exhibits: we carry out such characterisation in the next subsection.

3.2 Diagram characterising the equilibrium population's modes

In the last subsection we saw how a difference in the lifetimes of our two phenotypes can lead to a new type of equilibrium regime for the population, which consists of a hybrid between the classical low and high mutation regimes: this new regime exhibits both a local probability maximum (as in the high-mutation case) and a maximum at the boundary (as in the low-mutation regime).

Looking at the shapes of the equilibrium distributions in Figure 4, we see that these shapes can be well classified in terms of the number and nature of their stationary points, which in turn determine the number and location of the distribution maxima, or *modes*.

Figure 4(a) has only one stationary point, which is a minimum, and this implies the existence of two modes at $x = 0$ and $x = 1$ (the probability density in fact diverges to infinity at these boundary points, though this singularity does not affect the possibility of normalising the distribution): this characterises the regime of low mutation as one where

the population polarises about one of the phenotypes, and rarely switches to the other.

Figure 4(b) also has only one stationary point, which is a local maximum: this suggests that the dynamics of the population in this regime will typically be one where a mixed phenotypic state fluctuates around this maximum.

Figure 4(c) shows a novelty of the present model: we have two stationary points, a local maximum and a local minimum, which implies the existence of a second mode also at one of the boundaries. This suggests that the dynamics will tend to polarise the population around one specific phenotype: the existence of the local maximum, however, suggests that this state of polarisation should be periodically lost in favour of a mixed configuration for the two phenotypes, and that this switch should happen considerably more often than the switch between the polarised states of (a). This, however, can only be elucidated by considering the model's dynamics explicitly, and could be the topic of a future work.

In order to understand how parameter values relate to cases (a), (b) and (c), we use the probability distribution function we obtained in the last section to locate the stationary points of the distribution ϕ .

The condition

$$\frac{d\phi(x)}{dx} = 0,$$

which gives the stationary points, leads to the following rational equation for x :

$$\left(\alpha + \frac{a}{x} - \frac{b}{1-x}\right)(x + \lambda(1-x)) + 1 - \lambda = 0, \quad (3.3)$$

where

$$a = \lambda\theta - 1, \quad b = \frac{\theta}{\lambda} - 1, \quad \text{and} \quad \alpha = s + \theta\left(\frac{1}{\lambda} - \lambda\right).$$

Multiplying equation (3.3) by factors x and $(1-x)$, we obtain a polynomial equation of degree 3, which admits three solutions. We are, however, only interested in solutions lying on the real interval going from 0 to 1, since these correspond to meaningful values for the relative frequency x .

Rather than solving the cubic in x , we can use (3.3) to find a functional expression for $\theta = Nu$ (for which equation (3.3) is linear). By considering θ as a function of x , and

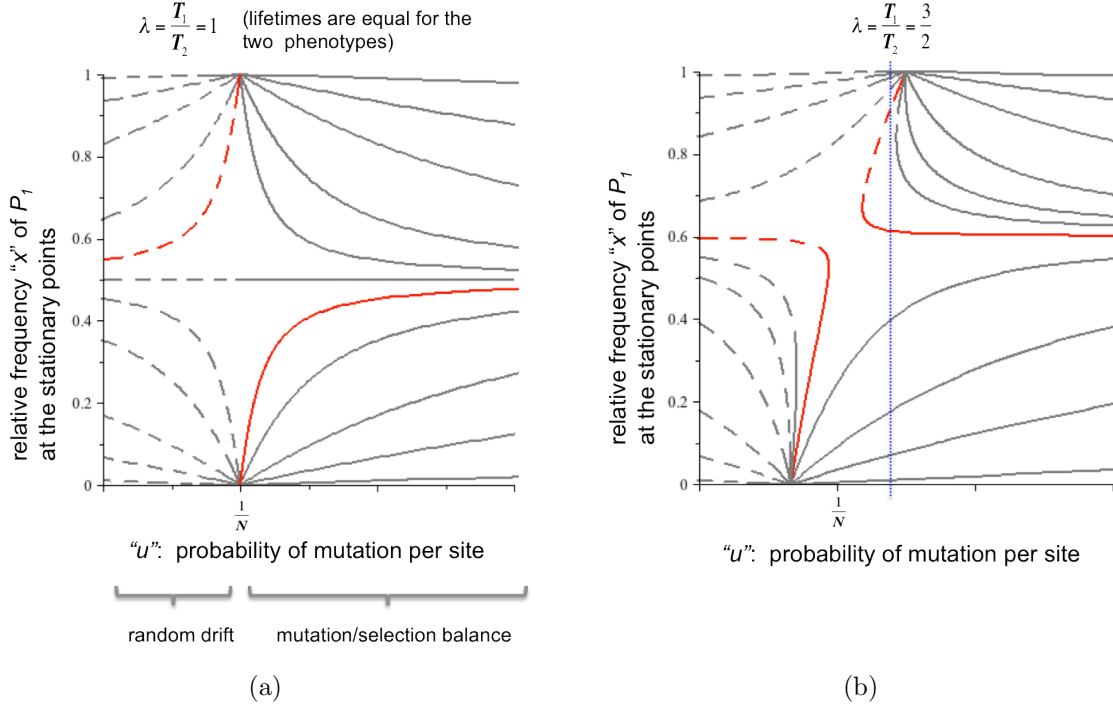


Figure 5: *Stationary points for the equilibrium distribution (a) in the classical case where $\lambda = T_1/T_2 = 1$, and (b) for $\lambda = T_1/T_2 = 3/2$. Each line corresponds to a different value of the selection coefficient " s ": dashed lines are for local minima, solid lines for local maxima. When $\lambda \neq 1$ bimodal equilibrium states exist: the dotted line corresponds to the value of the mutation probability " u " which gives rise to the distribution in Figure 4(c), for the value of the selection coefficient " s " corresponding to the line highlighted in red.*

looking at this function for different values of parameters s (reproductive selection) and λ (the lifetime ratio), we get a full characterisation of the distribution's stationary points and, as a consequence, of its modes: we do this in Figure 5.

Figure 5 shows the stationary points for the equilibrium distribution: the two pictures correspond to the two different cases $\lambda = 1$ and $\lambda \neq 1$. Different lines correspond to different values of the selection coefficient s , for which they give the dependence of the position of the stationary points on the mutation probability u . Dashed lines correspond to local minima and solid lines to local maxima.

Figure 5(a) shows the classical situation where $T_1 = T_2$ ($\lambda = 1$). As expected, we find

an abrupt transition in the nature of the stationary points when the mutation probability $u = 1/N$, at which value the equilibrium distribution turns from being U-shaped to being bell-shaped: however, the diagram shows that all parameter values except $u = 1/N$ lead to only one stationary point in the equilibrium distribution. Highlighted in red we see the functional dependence of the unique stationary point on the mutation probability, for a particular value of the reproductive selection coefficient s .

Figure 5(b) shows the same diagram for $\lambda = T_1/T_2 = 3/2$: we see highlighted in red the dependence of the equilibrium distribution's stationary points on the mutation probability, for the same value of reproductive selection s used in for the red curve in Figure 5(a). The diagram shows how near $u = 1/N$ there are regions where two stationary points coexist for the same distribution, a situation which is not encountered in classical population genetics models for haploid populations. The dotted line, which shows an example of this, corresponds to the regime in Figure 4(c).

An important fact which we learn from Figure 5 is that the diagram for $\lambda = 1$ is not robust with respect to changes in the parameter values, whereas it is robust for any other value of λ . This means that for any $\lambda \neq 1$ the diagram will exhibit regimes where the equilibrium distribution admits more than one stationary point, and the transition between the different qualitative types of equilibrium will come about through the same type of bifurcations which we see in Figure 5(b).

4 Estimation of $\lambda = \frac{T_1}{T_2}$

In this section we derive the parameter $\lambda = T_1/T_2$ from population data, and in particular from statistical quantities characterising the amount of neutral variation underlying phenotypes P_1 and P_2 . As mentioned above, in order to quantify the amount of neutral mutation we use the inbreeding coefficient concept, by defining the following two quantities

F_1 = probability that two organisms with phenotype P_1 have the same genotype,

F_2 = probability that two organisms with phenotype P_2 have the same genotype.

An important reason for using this approach is that it allows to extend an intuitive result obtained by Kimura and Crow in [8], where the equilibrium value for the inbreeding coefficient was computed for a population consisting of only one phenotype, rather than of two phenotypes like in our case.

The basic observation that allows Kimura and Crow’s calculation is that, if we denote the inbreeding coefficient for their unique phenotype by F , and if we assume the population changes according to Wright’s process, in the absence of mutation the value of F changes according to the following equation:

$$F(t+1) = \frac{1}{N} + \left(1 - \frac{1}{N}\right)F(t). \quad (4.4)$$

The intuitive reason for this is that at generation $t+1$ any two individuals have a probability $1/N$ of being born from the same parent: if they do, in the absence of mutation they share the same genotype with probability 1, which gives the first term on the left hand side of (4.4).

In the presence of mutation, assuming that each mutation generates a mutation which previously didn’t exist,

$$F(t+1) = \left\{ \frac{1}{N} + \left(1 - \frac{1}{N}\right)F(t) \right\} (1-u)^2,$$

which leads to the the equilibrium average value

$$\langle F \rangle = \frac{1-2u}{2Nu-2u+1} \approx \frac{1}{2Nu+1}. \quad (4.5)$$

The “infinite alleles” assumption, according to which each new mutation produces a genotype not contained in the population, is equivalent to assuming that the length L of our genotype is very large, and we shall be making the same assumption in order to generalise (4.5).

The line of reasoning used to obtain (4.5) can be extended to our situation, where a haploid population subdivided into two phenotypes P_1 and P_2 changes by single death-and-birth events, rather than at discrete generations: since this setting is less symmetric the details are more articulated, though the idea behind the calculation remains the same.

In our model the change in F_1 at time t depends 1) on whether a death has taken place, 2) on the phenotype of the organism that died, and 3) on the phenotype of the newborn (taking into account the possibility that a mutation might have taken place).

To reduce the complexity of the calculation we make the assumption that a newborn, before mutation, shares the same phenotype of the organism which has died. This would only strictly hold if the relative sizes for phenotypes P_1 and P_2 stayed a fixed throughout the process: in Figure 6 we show, however, the result of a simulation that suggests that our assumption leads to a formula which gives a rather precise approximation, and this is sufficient to support the paper's claim that it is theoretically feasible to extend the population-genetical characterisation of biological function.

To compute the change in F_1 we therefore need to consider three cases for the type of event taking place at time t :

- A:** an organism of type P_1 dies, is replaced by a newborn of the same type, and the newborn might mutate, either at the phenotypically-linked site, or at one of the neutral sites,
- B:** an organism of type P_2 dies, is replaced by a newborn of the same type, and the newborn might mutate, either at the phenotypically-linked site, or at one of the neutral sites,
- C:** no death takes place, so no replacement happens.

According to our process, and taking into account our simplifying assumption -that sets the phenotype of a dead organism equal to that of the subsequent newborn- the probabilities of events A , B and C are:

$$P(A) = \frac{x}{T_1}, \quad P(B) = \frac{1-x}{T_2}, \quad P(C) = 1 - \frac{x}{T_1} - \frac{1-x}{T_2}.$$

Keeping in mind that the quantity F_1 is defined as the probability that two organisms chosen at random from the population and which have phenotype P_1 also share the same genotype, we now need to find how F_1 changes in each of the three cases A , B and C .

Preliminary calculation of sampling probabilities:

The probability $F_1(t + 1)$ is associated with a couple of organisms drawn at random from the population, and therefore it depends on whether one of the two sampled organisms happens to be the one which was born during the last step. In particular, we need to distinguish the following three sampling events:

- S_1 : the newborn organism is chosen in the sampling, but its parent is not,
- S_2 : the sampled couple consists of the newborn and its parent,
- S_3 : the newborn is not sampled.

Assuming that the organisms are sampled from the population without reinsertion, the probabilities of events S_1 , S_2 and S_3 are as follows:

$$\begin{aligned} P(S_1) &= \frac{1}{X} + \frac{1}{X-1} - \frac{3}{X(X-1)}, \\ P(S_2) &= \frac{2}{X(X-1)}, \\ P(S_3) &= 1 - \frac{1}{X} - \frac{1}{X-1} + \frac{1}{X(X-1)}, \end{aligned}$$

where, like before, X is the total number of individuals with phenotype P_1 .

Using these probabilities we can now find $F_1(t + 1)$ in each of the three cases A , B and C

Case A:

To see how F_1 changes after an event of type A we need to know two things:

- whether the newborn mutated (and whether the mutation happened at the site linked to the change in phenotype),
- whether one of the two organisms which are selected at random to compute the probability F_1 is the newborn (and whether the other happens to be its parent organism).

If a mutation happens at the phenotypically-linked site, any two organisms of type P_1 sampled at time $t + 1$ will have the same probability of sharing the same genotype, as

they did at time t : since the probability of mutation at any site is u , this will contribute

$$u F_1(t)$$

to the average value of F_1 at time $t + 1$, conditional to event A .

If a mutation happens at a neutral site, the probability of the newborn having the same genotype as any of the other organisms is zero (this is a consequence of assuming that L is large enough for each neutral mutation to produce a totally new genotype). Since the probability of a neutral mutation is $(L - 1)u$, we have that in this case the contribution is

$$0 \cdot (L - 1)u \left(P(S_1) + P(S_2) \right) + F_1(t) \cdot (L - 1)u P(S_3) = F_1(t)(L - 1)u P(S_3),$$

The first term on the left-hand-side corresponds to the event that the newborn is chosen at the sampling (events S_1 and S_2 above), whereas the second term, which gives the non-zero contribution, corresponds to the fact that the probability F_1 remains unchanged as long as none of the chosen organisms is the newborn (event S_3).

Finally, for the case in which no mutation happens at any site, which has probability $(1 - Lu)$, we get the following contribution:

$$(1 - Lu) \{ F_1(t) \cdot (P(S_1) + P(S_3)) + 1 \cdot P(S_2) \},$$

where the second term corresponds to the fact that, as long as no mutation takes place, the probability that the newborn shares its genotype with its parent is equal to 1.

Therefore, if we denote the value of $F_1(t + 1)$ conditional to event A by $F_1(t + 1|A)$, summing all three contributions we get

$$F_1(t + 1|A) = u F_1(t) + F_1(t)(L - 1)u P(S_3) + (1 - Lu) \{ F_1(t) \cdot (P(S_1) + P(S_3)) + P(S_2) \}.$$

Case B :

In this case we have a newborn of type P_2 . Like for case A , we use the term $F_1(t + 1|B)$ to denote the new value of F_1 conditional to B , and we have that

$$F_1(t + 1|B) = \left(1 - u (P(S_1) + P(S_2)) \right) F_1(t).$$

It's straightforward to see why: if the newborn is of type P_2 , the inbreeding coefficient remains unchanged unless the newborn mutates in the phenotypically-linked site and is subsequently chosen in the sampling: the probability of this event is $u \cdot (P(S_1) + P(S_2))$.

Case C :

In this case nothing happens, so we have that

$$F_1(t+1|C) = F_1(t).$$

We can now write the value of $F_1(t+1)$ as the sum of the conditional contributions multiplied by their respective probabilities:

$$F_1(t+1) = F_1(t+1|A) P(A) + F_1(t+1|B) P(B) + F_1(t+1|C) P(C),$$

and we can use symmetry to obtain an analogous relation for F_2 .

In the limit of large N these two relations simplify substantially, so that up to order $1/N^2$ we get

$$\begin{aligned} F_1(t+1) - F_1(t) &= \frac{2}{N^2 T_1} \left\{ \frac{1}{x} - \frac{1}{x} F_1(t) \left(1 + \theta(\lambda(1-x) + x(L-1)) \right) \right\}, \\ F_2(t+1) - F_2(t) &= \frac{2}{N^2 T_2} \left\{ \frac{1}{1-x} - \frac{1}{1-x} F_2(t) \left(1 + \theta\left(\frac{1}{\lambda}x + (1-x)(L-1)\right) \right) \right\}, \end{aligned}$$

where the terms x and $(1-x)$ at the denominator arise from the sampling probabilities $P(S_1)$, $P(S_2)$, $P(S_3)$.

Therefore, denoting by $\langle \cdot \rangle$ the average with respect of all realisations of our process, we get the following relations linking the moments of the observable quantities to the model parameters:

$$\begin{aligned} \langle F_1/x \rangle + \theta \left(\lambda(\langle F_1/x \rangle - \langle F_1 \rangle) + (L-1)\langle F_1 \rangle \right) &= \langle 1/x \rangle, \\ \langle F_2/(1-x) \rangle + \theta \left(\frac{1}{\lambda}(\langle F_2/(1-x) \rangle - \langle F_2 \rangle) + (L-1)\langle F_2 \rangle \right) &= \langle 1/(1-x) \rangle. \end{aligned}$$

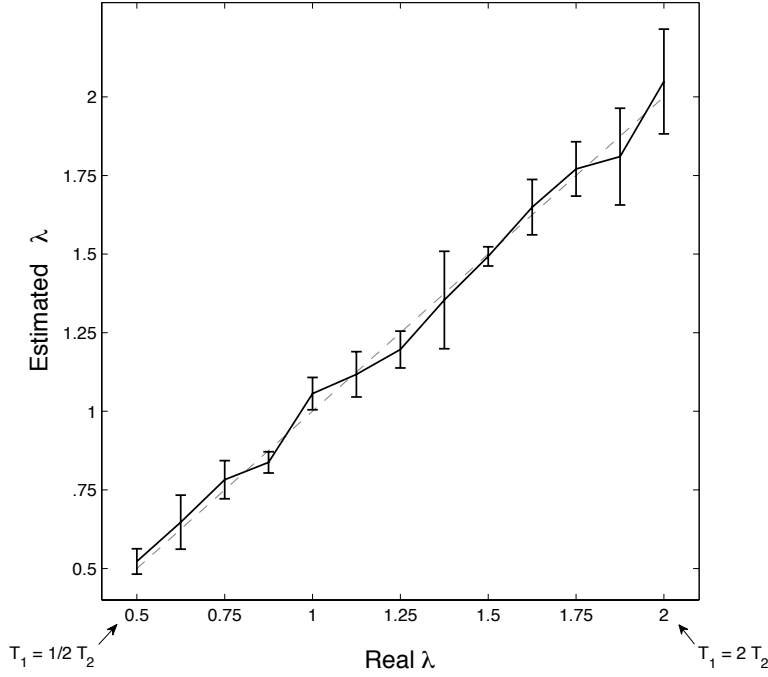


Figure 6: Comparison of the actual value of $\lambda = T_1/T_2$ and the value estimated from Eqn. (4.6), for simulations using values ranging from $\lambda = .5$ to $\lambda = 2$. The moments needed for Eqn. (4.6) are estimated from 10000 process realisations for each value of λ ; the other parameters are $s = -5$, $u = 0.007$, $N = 1000$ and $L = 40$.

Though the notation is somewhat cumbersome, it is easy to see that these two equations offer a relation between the model parameters θ and λ and the averages of the six random quantities

$$F_1, F_2, \frac{1}{x}, \frac{1}{1-x}, \frac{F_1}{x}, \frac{F_2}{1-x},$$

all six of which are in principle statistically observable.

Since this paper focuses on the parameter $\lambda = T_1/T_2$, in virtue of its putative relevance in terms of function, we proceed by solving both equations for θ , and equating them in order to find a relation for λ .

In order to express the mentioned relation in a more compact form we define the

following auxiliary quantities

$$R = \frac{\langle F_2 \rangle}{\langle F_1 \rangle} \cdot \frac{\langle 1/x \rangle - \langle F_1/x \rangle}{\langle 1/(1-x) \rangle - \langle F_2/(1-x) \rangle},$$

$$Q_1 = \frac{\langle F_1/x \rangle}{\langle F_1 \rangle} - 1, \quad Q_2 = \frac{\langle F_2/x \rangle}{\langle F_2 \rangle} - 1.$$

In terms of these quantities, the equation for λ takes the following form

$$R = \lambda \frac{\lambda Q_1 + L - 1}{Q_2 + \lambda(L - 1)},$$

and this relation leads to a quadratic equation that only admits one non-negative solution:

$$\lambda = \frac{1}{2Q_1} \left\{ (R - 1)(L - 1) + \sqrt{(R - 1)^2(L - 1)^2 + 4RQ_1Q_2} \right\}. \quad (4.6)$$

Figure 6 shows the result of using formula (4.6) to estimate λ , for a series series of simulations where the real value of λ ranges from .5 (i.e. $T_1 = 1/2 T_2$) to 2 (i.e. $T_1 = 2 T_2$): we see that the average values of such estimations are well aligned with the actual values.

The magnitude of the standard deviation for our estimations, on the other hand, is considerable, especially in view of the fact the 10000 realisations of the process were used to estimate each value of λ : it is clear that a substantial increase of efficiency will be needed to make the theory relevant to actual empirical phenomena.

This practical consideration should not obfuscate, however, the fact that equation (4.6) provides a relation between population statistics given by x , F_1 and F_2 , and parameter $\lambda = T_1/T_2$, which contains functional information related to a gene's effect on an organism's ability to survive, rather than on its reproductive fitness.

5 Outlook

We have shown that differentiating the lifetimes of two phenotypes independently from their fertility leads to a qualitative change in the equilibrium state of a population: since survival and reproduction are quite distinct macro-functions performed by any living organism, this contributes to extend the population-genetical characterisation of biological function.

We have furthermore shown that, by using information provided by neutral variation, the lifetime ratio λ can be expressed explicitly in terms of statistically observable quantities, and independently of all other parameters. This both gives some support to the possible empirical relevance of the proposed modelling approach, and suggests observable quantities that can be useful in characterising the stochastic equilibrium of a population in terms of the functional features of the individuals which comprise it.

It needs to be stressed, however, that the statistical resolution needed to estimate λ efficiently following this method seems to go beyond what could be achieved empirically: in order to obtain Figure 6, 10000 realisations of the system were needed for each parameter value, and for each value 5000 generations were needed for the population to relax to its stochastic equilibrium.

This study aims to be a proof of principle, and should only be considered a worst case scenario, which nevertheless shows that inferring functional details from population genetical considerations is a definite theoretical possibility. It is left for a future work to assess its practical feasibility by improving the estimation efficiency, possibly while considering dynamical statistics explicitly: it is useful to remember, however, that the dynamics of no system has ever been understood without a sufficient grasp of how relevant forces balance one another to allow observation.

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